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STANDARDISATION OF *CARISSA CARANDAS* LINN: A DRUG USED IN INDIAN SYSTEM OF MEDICINE AS PER W.H.O. GUIDELINES

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ABSTRACT

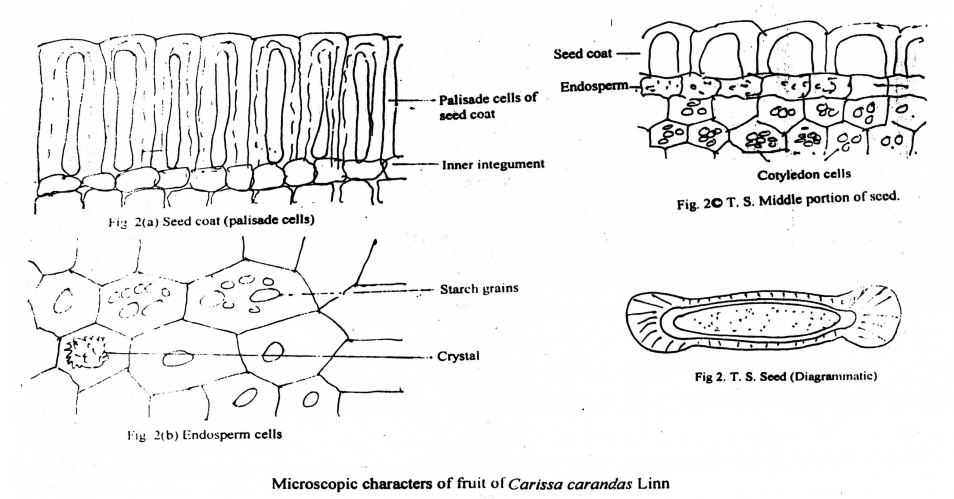
The standardisation of *Carissa carandas* were carried out as per Ayurvedic Pharmacopoeia of India, Pharmacopoeial standard for Ayuvedic Formulation as per the W.H.O. parameters of quality control for medicinal plants. The sample procured from local market. Fruit was found to be ovoid, to spherical, smooth having light green to pink color with sour taste. The physicochemical, toxicological and pharmacological parameters were determined. The sample was found contaminated with organopesticides and alderin. Qualitative analysis of plant metabolites like alkaloid, carbohydrates, protein, resins, starch have been carried out. Various Thin layer chromatography pattern of the powder in petroleum ether and methanol in different mobile phases were also reported.

KEYWORDS: *Carissa carandas*, Thin layer chromatography, Ayuvedic Formulation, W.H.O. parameters

INTRODUCTION

Carissa carandas Linn (Apocynaceae) is an evergreen diffuse and spiny shrub occurring through out the country. The plant is very valuable for the Indian System of medicine particularly Ayurveda. It is used for alleviating vata and pitta disorders. Its fruits and seed latex are used for treating rheumatoid arthritis, anorexia, indigestion, colic, hepatomegaly, splenomegaly, piles, cardiac diseases, oedema, amenorrhoea, fever and nervine disorder (Pushpangadan., 2003). The roots are useful in stomach disorder, intestinal worms, Scabies, diabetic, ulcer and pruitis (Sharma et al., 2001). Alcoholic extract of the root exhibits cardiotonic effect (Vohra and De., 1963). The plant is also useful to bring down blood pressure (Chunekar., 1982). Various fatty acids such as palmitic (66.42%), stearic (9.36%), Oleic (2.04%) and linoleic (0.99%) acids were found in seed of Carissa carandas (Shrivastava and Bakodia., 1979). A terpinic alcohol carisol (1), which is an epimer of α-amyrin was isolated from its fresh fruits. Glucose and galactose as well as the amino acids serine, glutamine, alanine, valine, phenylalanine and glycerin reported in this study correlate with the previous studies (Zafar et al., 1985)

Carissa carandas edible flesh finds an important place in the India recipes particularly puddings and jellies. It is at large scale by means of agricultural practices. Therefore, the possibility of contamination due to use pesticides and fertilizer cannot be ignored. Considering the prevailing agro-forestry technique and its therapeutic potential the standardization of Carissa carandas were under taken the assessing the quality in accordance to international guidelines.



Microscopic characters of fruit of *Carissa carandas* Linn

Table-I: The Topographical details of collection of fruit of *Carissa carandas* Linn.

Place of collection	Buxi ka Talab (Lucknow) (Uttar Pradesh, INDIA)
Time of collection	July, 2002
Location	26.55°N and 80.59°E
Climate	Subtropical, dry, winter, hot summer and followed by heavy rains.
Rainfall average	40-100cm (Annual)

micro-techniques (Kay., 1938 ; Johnsen., 1940) and representative diagram were sketched through Lucida camera. For study of isolated cells and tissues, small pieces of different fruit were macerated with schult's fluid, washed and mounted in glycerin. Physio-chemical test were carried out adopting, standard procedure (Trease and Evan., 1983 ; Kokate and Purohit., 1997). Ash-value, solubility (cold percolation and graded) in the various solvents such as PE, alcohol (95%) and water values, screening of thin layer chromatography, effect of different chemical reagents and florescence analysis under ultra-violet radiation which are considered to be immense help in detection of adulterants were also carried out (Chase and Pratt., 1949 ; Kokoski *et al*., 1958; API ' 1999; PSAF, 1987; Winton and Winton., 2001). Qualitative for identification of phytoconstiuents like alkaloids, steroids, terpenoids, phenols, tannins, saponins and flavinoids etc were also carried out (Kokate and Purohit., 1979).

Pesticides Analysis

Organochlorides pesticides were extracted from powdered drug of Cassia carandus adopting the procedure mentioned in WHO guidelines (WHO, 1998). The external organochlorides pesticides standard and sample extract were applied on GLC (Nucon 5767) with the following conditions. Column 6" x 1/8" (ID) glass column filled with 80-100 mess gas Chrom coated with mixture of 1.5% of OV-17 AN d1.95 OV210, temperature 190°C, Detector Ni63ECD, injector tempreture 250ºC. carrier gas IOLARgrade, Nitrogen flow rate 60ml/min. using the above mentioned analytical condition of the level of detection (LOD) and level of the quantitation (LOQ) of the organochlorides pesticides were in the range 0.1-0.5µg/L and 1-3µg/L respectively. Recoveries in the fortified sample were found to be 80-95%.

METHODS

Fruit of *Carissa carandas* were purchased from local market, Lucknow U.P (INDIA). The topographical details of collection are summarized in Table-I, microtome section were taken, stained and mounted following the usual plant

TLC Analysis

Various extracts of *Carissa carandas* were subjected to TLC on silica gel-G (manually coated on glass plate in laboratory). Various combinations of solvents of different polarity were adjusted to found out the suitable TLC pattern of drug. The resolution pattern was detected in iodine chamber and LB reagent. The results are summarized in table - II.

Table-II: Thin Layer Chromatographic pattern of extract of *Carissa carandas* Linn. (Fruit).

S.No	Stationary Phase	Solvent System	Loading Extract	Visualisation/ Detection	R_f Value (R_f x 100)
1.	Silica gel-G	C_6H_6:MeOH :Toluene (5:20:2)	MeOH (graded)	I_2	35.0, 50.0, 60.7, 71.0
2.	Silica gel-G	Hexane: $CHCl_3$: MeOH (20:5:5)	MeOH (graded)	I_2	17.0, 35.0, 53.0
3.	Silica gel-G	C_6H_6:EtOAc:PE (3:2:1)	PE (graded)	I_2	50.0, 75.0, 80.0
4.	Silica gel-G	C_6H_6:MeOH (5:1)	PE (Graded)	LB	22.2, 59.3, 66.7, 70.4, 74.0, 77.8
5.	Silica gel-G	C_6H_6:MeOH (3:20)	$CHCl_3$ (Graded)	LB	80.7, 85.0, 87.9, 89.3
6.	Silica gel-G	……do…….	EtOAc (Graded)	LB	62.9, 85.0, 87.9, 89.3
7.	Silica gel-G	C_6H_6:EtOAc:MeOH:AcOH (5:2:10:5drop)	MeOH (graded)	LB	28.0, 30.0, 40.0, 70.0, 95.0
8.	Silica gel-G	MeOH: Toluene (20:5:2)	EtOAc (Graded)	LB	70.5, 78.8, 84.2, 89.0,
9.	Silica gel-G	Toluene:MeOH (2:2:1)	EtOAc (Graded)	LB	25.0, 40.0, 54.0, 95.0

Table-III: Organoleptic identification of *Carissa carandas* Linn

Parameters	Observations
Botanical	
Sensory Evaluation	Ovoid, to spherical, 1-1.5 cm across, 2-4 seeds
Visual macroscopy	Smooth
Touch	Characteristic
Odour	Sour
Taste	Light green to pink
Colour	
Foreign Organic Matter	No adulterants have been found

RESULTS

Description of Fruit

Fruit an ovoid in vary ½ inch long. Seeds 2-4, necked the unripe fruit sour in taste, colour of fruit very light green to pink.

Table-IV: Qualitative analysis of plant metabolites (primary and secondary both) of fruit of *Carissa carandas* Linn .

S. No	Phytochemicals	*Carissa carandus* (Locally Collected Sample)
1.	*Alkaloid*	+ve
2.	Carbohydrate	+ve
3.	Flavonoid	-ve
4	Protein	+ve
5.	Resin	+ve
6.	Saponin	+ve
7.	Starch	+ve
8.	Steroids	+ve
9.	Tannin	+ve
10.	Triterpenoids	+ve

Key: +ve – Constituent is present

Table-V: Physico-chemical Parameters of fruit of *Carissa carandas* Linn.

S. No.	Parameters	Observations
1.	Physicochemical	
	Ash Values (% w/w)	
	a) Total Ash Value	4.80±0.25
	b) Acid Insoluble Ash	1.17±0.28
	c) Water Soluble Ash	1.67±0.15
	Extractive values (% w/w)	
	Cold percolation method	
	PE (40-60°)	5.16±0.41
	EtOH (95%)	36.5±1.02
	H_2O	39.66±1.03
	Soxhlet graded extraction method	
	PE (40-60°)	10.05±0.57
	EtOH (95%)	41.08±1.23
	H_2O	15.13±1.39
	Moisture content (fresh fruit)	16.65±0.15 (% w/w) By Hot air Oven method 16.28 ± 0.15 (%v/w) By Azeotropic method
	Volatile Oils	Traces
2.	Pharmacological	
	Swelling Index	1.92 ± 0.14
	Foaming Index	333
3.	Toxicological	
	Pesticides (ppm)	
	α-HCH	0.45
	β-HCH	0.38
	γ-HCH	0.72
	δ-HCH	1.51
	Total HCH	3.06
	Aldrin	0.64
	DDT	ND

Values presented are mean of triplicate (Mean ± s. d.).; ND- Not detected

Table-VI: Behaviour of fruit powder of *C. carandas* with different reagents observed under ordinary light and UV-radiation.

S. NO.	Interaction of *C. carandus* powder with different reagent	Colour produced under ordinary light	Colour produced under UV-radiation	
			Short (254nm) wavelength	Long (366nm) wavelength
1.	Drug (P) as such	Light Brown	Yellowish	Orange
2.	P+ Nitrocellulose in amyl acetate	Reddish brown	Dark brown	Grey
3.	P+1N.NaOH in water	Dark Yellow	Brown	Black
4.	P+1N.NaOH+ Nitrocellulose in amyl acetate	green	Brown	Green
5.	P+1N.HCL+Nitrocellulose in amyl acetate	Yellowish green	yellowish green	Dark green
6.	P+1N.NaOH in Methanol	Brown	Pink	Brown
7.	P+50%KOH	Brown	Dark Pink	Green
8.	P+1N.HCL	Dark red	Brown	Light brown
9.	P+50%H_2SO_4	Light Brown	Violet	Dark brown
10.	P+50%HNO_3	brick red	Pink	Green
11.	P+Conc.HNO_3	dark brown	Dark Brown	Light Brown
12.	P+ Acetic acid	brown	brown	Brown
13.	P+Conc. H_2SO_4	Red	Red	Dark red
14.	P+ Iodine water	Dark Pink	Brown	Dark brown

Powder Description
Creamish brown in colour, epidermal cells of epip-parenchyma with cell content, mesocarp cells, stone cells, rossete crystals and starch grains.

a. Macroscopic
Barriers are spherical or ellipsoidal, purple, 1-1.5 cm across, 4-6 seeded. The results are summarized in Table-III.
b. Microscopic
Fruit
T.S. of fruit (Fig-Ia &Id) shows single layer of square shaped epidermis of pericarp, followed by to barrel shaped, 4-6 layer of hypodermal cells. Epidermis is covered by cuticle brownish in colour. Hypodermis cells contains cell contents, rossete crystal of calcium oxalate and starch grains. Mesocarpic region almost consist of larged thin walled oval to polygonal parenchymatouscells. Some cells contain cell content and rossete crystals. The laticiferrous channel or duct appear in the region are elongated tubular. Vascular strands are also present in this region. Endocarpic region is 2-3 layers of thick walled cells (fig -1).

T.S. of seed (fig- 2a-2c) shows tangitially elongated cells, outer integument of both sides elongated and also. Palisade cells which are thick walled and brownish in colour. Inner is also brownish in colour and followed by few layers of endosperm. The cotyledon tissue consists of aileron grains and rounded starch grains and some of cotyledon has cell contents. Cells are compactly arranged (fig- 2).

DISCUSSION

The drug powder is hygroscopic in nature having major plant metabolites except steroid and flavinoids (Table-IV). The moderate foaming index (333) of *Carissa carandas* confirm presence of appreciable amount of saponins. A value of swelling index (1.92) indicates that the amount of mucilage, pectin and hemicellulose are present in low quantity (Table-V). The perusal of table-V reveals the abundance of polar phytochemicals with high extractive values polar solvents such as ethanol and water. The acid insoluble ash is the indicative of low amount of non physiological contamination. Presence of various HCH isomers and alderin indicates that the fruits of are contaminated with HCH. It is due excessive use of these pesticides in India and banned after 1997. The higher value of isomers in the sample has a consequence of regular used of insecticides formulations containing linden (HCH) as the active ingredient. Behaviour of drug powder in different reagents were observed under ordinary light and UV radiation (254 and 366nm (Table-VI). None of reagent shows the diagnostic colour reaction under either ordinary or UV radiation.

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